

REMARKS/ARGUMENTS

The foregoing amendments in the specification and claims are of formal nature, and do not add new matter.

Prior to the present amendment, Claims 58-65, 68-70 and 74-77 were pending in this application. With this amendment, Claims 74 and 76 have been amended to clarify what Applicants have always regarded as their invention and new Claims 78-85 have been added.

Claims 58-65, 68-70 and 74-85 are pending after entry of the instant amendment.

Support for new Claims 78-81 can be found at least in Example 126, starting on page 351, line 18 of the specification. Support for new Claims 82-85 can be found at least in Example 117, starting on page 347, line 28 of the specification.

Priority

Applicants rely on the proliferation of rat utricular supporting cells assay (Example 116) for patentable utility which was first disclosed in PCT/US99/05028 filed on March 8, 1999, priority to which has been claimed in this application. Accordingly, the present application is entitled to at least the March 8, 1999 priority for subject matter defined in Claims 59-65, 68-70 and 74-77. In support, Applicants enclose herewith page 277, describing the proliferation of rat utricular supporting cells assay (Example 116) of the PCT Publication WO 99/46281, corresponding to PCT Application PCT/US99/05028.

Applicants further rely on the chondrocyte re-differentiation assay (Example 126) and the glucose/FFA uptake assay (Example 117) for patentable utility which was first disclosed in PCT/US00/04341 filed on February 18, 2000, priority to which has been claimed in this application. Accordingly, the present application is entitled to at least the February 18, 2000 priority for subject matter defined in Claims 63-65, 68-70 and 74-85. In support, Applicants enclose herewith page 359, describing the chondrocyte re-differentiation assay (Example 126), and page 355-356, describing the glucose/FFA uptake assay (Example 117), of the PCT Publication WO 00/53756, corresponding to PCT Application PCT/US00/04341.

Withdrawn Objections and/or Rejections

Applicants note and appreciate the withdrawal of the earlier objections and rejection.

Claim Rejections Under 35 U.S.C. §101

(A) The Examiner asserts that Claim 76, "as written, does not sufficiently distinguish over cells as they exist naturally because the claims do not particularly point out any non-naturally occurring differences between the claimed products and naturally occurring products."

Without acquiescing to the Examiner's position in the current rejections, and without prejudice to further prosecution of the subject-matter in one or more continuation or divisional applications, Applicants have amended Claim 76 (and, as a consequence, those claims dependent from the same) to recite an "isolated host cell." Thus, the claimed cells are distinguished over cells in nature. Hence, Applicants respectfully request reconsideration and withdrawal of the present rejection.

(B) Claims 58-62 and 74-77 are rejected under 35 U.S.C. §112, second paragraph, for allegedly "being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." The Examiner notes that Claims 58-62 and 71-74 are indefinite for reciting "'wherein the nucleic acid encodes a polypeptide that is a mitogen for inner ear supporting cells' in Claims 58-62 because it is unclear which nucleic acid the phrase refers to."

Without acquiescing to any of the rejections, Applicants submit that the cancellation of Claim 58 renders the rejection of this claim moot. Further, Claims 59-62 (and, as a consequence, those claims dependent from the same) have been amended to recite an isolated nucleic acid encoding a polypeptide having at least 85-99% sequence identity to the *amino acid sequence* of (a) or (b) or (c) or (d) or (e). Therefore, the phrase "wherein the nucleic acid encodes a polypeptide that is a mitogen for inner ear supporting cells" clearly refers to the isolated nucleic acids having at least 85-99% sequence identity wherein these nucleic acids encode polypeptides that are mitogens for inner ear supporting cells. Accordingly, Applicants respectfully request the Examiner to reconsider and withdraw the present rejection.

Claim Rejections Under 35 U.S.C. §102(b)

Claims 58 and 74-77 are rejected under 35 U.S.C. §102(b) as being anticipated by Struyk *et al.* (*The Journal of Neuroscience*, 15(3):2141-2156, March 1995). The Examiner alleges that

"Struyk et al. teach a polynucleotide sequence having at least 80% identity with a nucleic acid sequence encoding the polypeptide of SEQ ID NO:523."

Without acquiescing to either of these rejections, Applicants submit that the cancellation of Claim 58 renders the rejection of this claim moot. Furthermore, Claim 74 has been amended to be dependent on Claim 59. Accordingly, Applicants respectfully request that the rejection of Claims 74-77 under 35 U.S.C. §102(b) as being anticipated by Struyk *et al.* be withdrawn.

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned attorney at the telephone number shown below.

Please charge any additional fees, including fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-2630 P1C66**).

Respectfully submitted,

Date: November 8, 2004

By: _____


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removal from the incubator, the plates were placed on the vortex mixer with the microtiter adapter head and vortex on the #2 setting for one minute. 80 μ l of the lysate were removed and added to the bDNA wells containing the Capture Hybridization Buffer, and pipetted up and down to mix. The plates were incubated at 53°C for at least 16 hours.

On the next day, the second part of the bDNA kit protocol was followed. Specifically, the Plates were removed from the incubator and placed on the bench to cool for 10 minutes. The volumes of additions needed were calculated based upon information provided by the manufacturer. An Amplifier Working Solution was prepared by making a 1:100 dilution of the Amplifier Concentrate (20 fm/ μ l) in AL Hybridization Buffer. The hybridization mixture was removed from the plates and washed twice with Wash A. 50 μ l of Amplifier Working Solution were added to each well and the wells were incubated at 53°C for 30 minutes. The plates were then removed from the incubator and allowed to cool for 10 minutes. The Label Probe Working Solution was prepared by making a 1:100 dilution of Label Concentrate (40 pmoles/ μ l) in AL Hybridization Buffer. After the 10 minutes cool down period, the amplifier hybridization mixture was removed and the plates washed twice with Wash A. 50 μ l of Label Probe Working Solution were added to each well and the wells were incubated at 53°C for 15 minutes. After cooling for 10 minutes, the Substrate was warmed to room temperature. Upon addition of 3 μ l of Substrate Enhancer to each ml of Substrate needed for the assay, the plates were allowed to cool for 10 minutes, the label hybridization mixture was removed, and the plates were washed twice with Wash A and three-times with Wash D. 50 μ l of the Substrate Solution with Enhancer were added to each well. The plates were incubated for 30 minutes at 37°C and RLU read in an appropriate luminometer.

The replicates were averaged and the coefficient of variation was determined. The measure of activity of the fold increase over the Protein 32 (buffer control) value indicated by chemoluminescence units (RLU). Samples which showed an at least two-fold value over the Protein 32 value were considered positive. PRO938 was positive in the above assay.

EXAMPLE 116: Proliferation of Rat Utricular Supporting Cells

In an effort to identify PRO polypeptides that act as potent mitogens for inner ear supporting cells which are hair cell progenitors (related to auditory hair cell regeneration), various PRO polypeptides were tested in the following assay.

The rat utricular epithelial cell line (UEC-4 cells) are aliquoted into 96 well plates with a density of 3000 cells/well in 200 μ l of serum-containing medium at 33°C. After overnight, the cultures are switched to serum-free medium at 37°C and the PRO polypeptide samples are added at various dilutions. After 24h incubation, ³H-thymidine (1 μ Ci/well) is added to the cultures for an additional 24h. The cells are then harvested using a Tomtec cell harvester. Because the epithelial cells are grown on a polylysine substrate, trypsin (1 mg/ml) is added to the culture wells for 30 min at 37°C to lift the cells before cell harvest. Cpm/well are counted with a matrix 9600 gas counter (Packard Instrument Company, Downers Grove, IL). Data is collected from 3 culture wells from each of the experimental groups and expressed as mean \pm SEM. A two-tailed, unpaired t-test is used for statistical analysis, as compared to the control group (treatment with TGF- α).

Average cpm counts which are at least 30% higher than the control values are considered positive for the assay. The following PRO polypeptides were positive in this assay: PRO337, PRO363 and PRO1012.

medium and added to the cells. Control samples are serum-free medium alone. On day 4, 20 μ l of the Cell Titer 96 Aqueous one solution reagent (Progenia) was added to each well and the colorimetric reaction was allowed to proceed for 2 hours. The absorbance (OD) is then measured at 490 nm. A positive in the assay is anything that gives an absorbance reading which is at least 15% above the control reading.

The following polypeptide tested positive in this assay: PRO200, PRO363, PRO731, PRO534, PRO866
5 and PRO1031.

EXAMPLE 125: Pericyte c-Fos Induction (Assay 93)

This assay shows that certain polypeptides of the invention act to induce the expression of c-fos in pericyte cells and, therefore, are useful not only as diagnostic markers for particular types of pericyte-associated
10 tumors but also for giving rise to antagonists which would be expected to be useful for the therapeutic treatment of pericyte-associated tumors. Specifically, on day 1, pericytes are received from VEC Technologies and all but 5 ml of media is removed from flask. On day 2, the pericytes are trypsinized, washed, spun and then plated onto 96 well plates. On day 7, the media is removed and the pericytes are treated with 100 μ l of PRO
15 polypeptide test samples and controls (positive control = DME+5% serum +/- PDGF at 500 ng/ml; negative control = protein 32). Replicates are averaged and SD/CV are determined. Fold increase over Protein 32 (buffer control) value indicated by chemiluminescence units (RLU) luminometer reading verses frequency is plotted on a histogram. Two-fold above Protein 32 value is considered positive for the assay. ASY Matrix: Growth media = low glucose DMEM = 20% FBS + 1X pen strep + 1X fungizone. Assay Media = low
20 glucose DMEM +5% FBS.

The following polypeptides tested positive in this assay: PRO200.

EXAMPLE 126: Chondrocyte Re-differentiation Assay (Assay 110)

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders
25 such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm² in Ham F-12 containing 10% FBS and 4 μ g/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100 μ l of the same media without serum and 100 μ l of the test PRO polypeptide, 5
30 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 μ l/well. After 5 days of incubation at 37°C, a picture of each well is taken and the differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO200, PRO285, PRO337, PRO526, PRO362,
35 PRO363, PRO531, PRO1083, PRO862, PRO733, PRO1017, PRO792, PRO788, PRO1008, PRO1075, PRO725 and PRO1031.

prepared by making a 1:100 dilution of Label Concentrate (40 pmoles/ μ l) in AL Hybridization Buffer. After the 10-minute cool-down period, the amplifier hybridization mixture was removed and the plates were washed twice with Wash A. 50 μ l of Label Probe Working Solution was added to each well and the wells were incubated at 53°C for 15 minutes. After cooling for 10 minutes, the Substrate was warmed to room temperature. Upon addition of 3 μ l of Substrate Enhancer to each ml of Substrate needed for the assay, the plates were allowed to cool for 10 minutes, the label hybridization mixture was removed, and the plates were washed twice with Wash A and three times with Wash D. 50 μ l of the Substrate Solution with Enhancer was added to each well. The plates were incubated for 30 minutes at 37°C and RLU was read in an appropriate luminometer.

The replicates were averaged and the coefficient of variation was determined. The measure of activity of the fold increase over the negative control (Protein 32/HEPES buffer described above) value was indicated by chemiluminescence units (RLU). The results are considered positive if the PRO polypeptide exhibits at least a two-fold value over the negative buffer control. Negative control = 1.00 RLU at 1.00% dilution. Positive control = 8.39 RLU at 1.00% dilution.

The following PRO polypeptides tested positive in this assay: PRO938, PRO200, PRO865, PRO788 and PRO1013.

EXAMPLE 116: Proliferation of Rat Utricular Supporting Cells (Assay 54)

This assay shows that certain polypeptides of the invention act as potent mitogens for inner ear supporting cells which are auditory hair cell progenitors and, therefore, are useful for inducing the regeneration of auditory hair cells and treating hearing loss in mammals. The assay is performed as follows. Rat UEC-4 utricular epithelial cells are aliquoted into 96 well plates with a density of 3000 cells/well in 200 μ l of serum-containing medium at 33°C. The cells are cultured overnight and are then switched to serum-free medium at 37°C. Various dilutions of PRO polypeptides (or nothing for a control) are then added to the cultures and the cells are incubated for 24 hours. After the 24 hour incubation, 3 H-thymidine (1 μ Ci/well) is added and the cells are then cultured for an additional 24 hours. The cultures are then washed to remove unincorporated radiolabel, the cells harvested and Cpm per well determined. Cpm of at least 30% or greater in the PRO polypeptide treated cultures as compared to the control cultures is considered a positive in the assay.

The following polypeptide tested positive in this assay: PRO337, PRO363 and PRO1012.

EXAMPLE 117: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a

sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as stimulators of glucose and/or FFA uptake in this assay: PRO181, PRO200, PRO337, PRO362, PRO363, PRO731, PRO534, PRO1114 and PRO1075.

The following PRO polypeptides tested positive as inhibitors of glucose and/or FFA uptake in this assay: PRO195, PRO322, PRO862, PRO868, PRO865 and PRO162.

10 EXAMPLE 118: Detection of Polypeptides That Affect Glucose and/or FFA Uptake in Skeletal Muscle (Assay 106)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by skeletal muscle cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by skeletal muscle would be beneficial including, for example, diabetes or hyper- or hypo-insulinemia.

15 In a 96 well format, PRO polypeptides to be assayed are added to primary rat differentiated skeletal muscle, and allowed to incubate overnight. Then fresh media with the PRO polypeptide and +/- insulin are added to the wells. The sample media is then monitored to determine glucose and FFA uptake by the skeletal muscle cells. The insulin will stimulate glucose and FFA uptake by the skeletal muscle, and insulin in media without the PRO polypeptide is used as a positive control, and a limit for scoring. As the PRO polypeptide being
20 tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as either stimulators or inhibitors of glucose and/or FFA uptake in this assay: PRO181, PRO200, PRO1083, PRO865, PRO162, PRO1008 and PRO1330.

25 EXAMPLE 119: Stimulation of Heart Neonatal Hypertrophy (Assay 1)

This assay is designed to measure the ability of PRO polypeptides to stimulate hypertrophy of neonatal heart. PRO polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of various cardiac insufficiency disorders.

Cardiac myocytes from 1-day old Harlan Sprague Dawley rats were obtained. Cells (180 μ l at 7.5×10^4 /ml, serum <0.1%, freshly isolated) are added on day 1 to 96-well plates previously coated with DMEM/F12 + 4% FCS. Test samples containing the test PRO polypeptide or growth medium only (negative control) (20 μ l/well) are added directly to the wells on day 1. PGF (20 μ l/well) is then added on day 2 at final concentration of 10^{-6} M. The cells are then stained on day 4 and visually scored on day 5, wherein cells showing no increase in size as compared to negative controls are scored 0.0, cells showing a small to moderate increase in size as compared to negative controls are scored 1.0 and cells showing a large increase in size as compared to negative
35 controls are scored 2.0. A positive result in the assay is a score of 1.0 or greater.

The following polypeptides tested positive in this assay: PRO195, PRO200, PRO526 and PRO792.